

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
19 May 2005 (19.05.2005)

PCT

(10) International Publication Number
WO 2005/045073 A1

(51) International Patent Classification⁷: **C12Q 1/68**

(21) International Application Number:
PCT/KR2003/002407

(22) International Filing Date:
10 November 2003 (10.11.2003)

(25) Filing Language: English

(26) Publication Language: English

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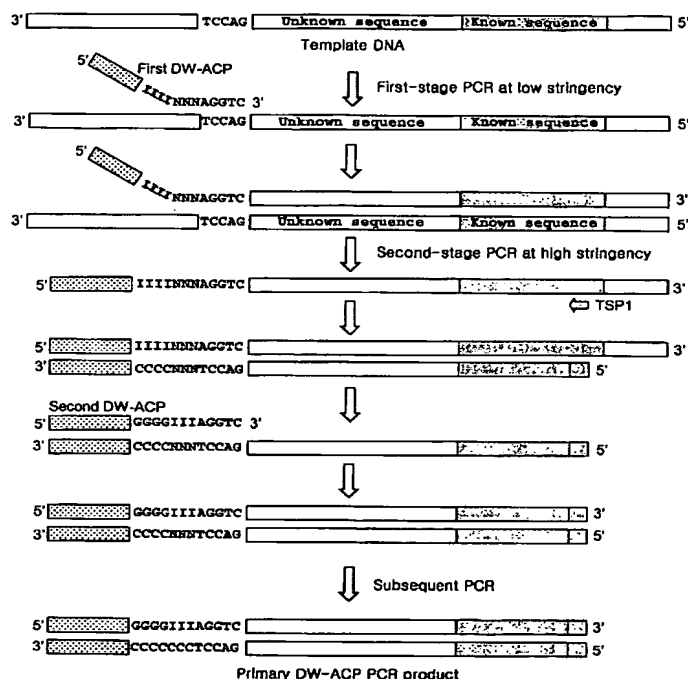
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(81) Designated States (*national*): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,
CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE,
GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR,
KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK,
MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT,
RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR,
TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),
Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE,
ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO,
SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM,
GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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(54) Title: METHOD FOR AMPLIFYING UNKNOWN DNA SEQUENCE ADJACENT TO KNOWN SEQUENCE



□ Known sequence
□ Unknown sequence
□ Universal sequence
⇐ Target Specific Primer
x Deoxyinosine

(57) Abstract: The present invention relates to a method for amplifying an unknown nucleotide sequence adjacent to a known nucleotide sequence, which comprises the step of (a) performing a primary amplification of said unknown nucleotide sequence using a DNA walking annealing control primer (DW-ACP) and a first target-specific primer; in which said step (a) comprises: (a-1) performing a first-stage amplification of said unknown nucleotide sequence at a first annealing temperature, comprising at least one cycle of primer annealing, primer extending and denaturing using a first degenerate DW-ACP containing a degenerate random nucleotide sequence to hybridize with said unknown nucleotide sequence and a hybridizing nucleotide sequence substantially complementary to a site on said unknown nucleotide sequence; and (a-2) performing a second-stage amplification at a second annealing temperature to render said first degenerate DW-ACP not to function as a primer.



Published:

— with international search report

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